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ANOPHELINE SURVEYS.

METHODS OF CONDUCT AND RELATION TO ANTIMALARIAL WORK.

By R. H. von Ezdorf, Surgeon, United States Public Health Service.

In the course of malarial investigations conducted by the United States Public Health Service since June 27, 1913, a number of surveys have been made with a view to studying the prevalence of malarial fevers and the conditions contributing thereto.

With reference to the Anopheline mosquitoes, these surveys have comprised chiefly an examination of the location and extent of their breeding places, and the determination of the species, their flight and habits.

For the intelligent application of antimalarial measures and the economic expenditure of public moneys, researches must be made at each place along the lines to be described.

No new method of procedure has been evolved, but the following description, together with the experiences encountered as a result of surveys made by the different working parties in 16 places in Arkansas, 2 in Mississippi, 1 in Alabama, 3 in Virginia, 2 in Georgia, 4 in South Carolina, and 1 in North Carolina, may serve as a guide to local health officers in undertaking similar work.

The local health officer should have information regarding the cases of malaria, the types of infection and seasonal prevalence in the locality to be studied. Other desirable information is the location and kind of industries, form of water supply and drainage system, topographical and meteorological conditions, including location and number of natural water courses, natural direction of surface drainage, presence of ditches, low, poorly drained areas, temperature, rainfall, and direction of prevailing winds.

The Malaria-Bearing Mosquitoes.

One must, of course, be familiar with the characteristic appearance of Anopheline mosquitoes in their various stages of development, so that a brief description of these may be warranted.

The malaria-bearing mosquitoes belong to the subfamily of Anophelinæ, which has been divided into 18 genera, but in a more recent nomenclature all of these are classed in one genus of Anopheles.

Many species have been determined, of which number 40 are more or less definitely known to act as hosts for the transmission of the malarial parasite.

Frederick Knab gives a list of 34 species of American Anopheles, of which number 8 have been definitely shown to serve as hosts for the malarial parasite. These are:

Anopheles albimanus.
argyritarsis.
crucians.
intermedium.

Anopheles quadrimaculatus.
pseudomaculipes.
pseudopunctipennis.
tarsimaculata.

The most common species encountered in the Southern States where surveys have been made are *Anopheles crucians, quadrimaculatus* and *punctipennis.* The last named, *Anopheles punctipennis,* is not considered to be a transmitter of malaria. *Anopheles crucians,* according to experiments by Beyer and his coworkers, is said to be a transmitter of the estivo-autumnal parasite only, but this needs confirmation. It is evident from this that Anopheles mosquitoes may be present, and yet no malaria prevail, and again, the prevailing species may determine the type of malaria. *Anopheles quadrimaculatus* will transmit all three types of malarial parasites, and this species is the one most commonly found in the Southern States where malaria prevails.

For the determination of the species, it is advised that specimens be collected and sent to a competent entomologist or person familiar with the characteristics of the Anophelines. Specimens collected by the working parties were sent for determination to Dr. L. O. Howard, Consulting Entomologist of the Public Health Service.

General Description.

The Anopheles mosquitoes pass, as do all mosquitoes, through the stages of (a) egg or ovum, (b) larva or wiggler, (c) pupa or tumbler, from which (d) the imago or mosquito emerges. Although the larval and pupal stages are passed in water, mosquitoes, during these stages, are, nevertheless, true air breathers. All stages present characteristics with which the health officer should be familiar.

The eggs of the Anopheles are minute and appear lying singly and flat on the water surface. Where present in numbers they frequently form symmetrical patterns. The individual egg is long and narrow, flat on one side, convex on the opposite side, and has lateral floats. (See figs. 1, 2, and 3.)

In making a survey of breeding places eggs are not looked for, although their presence is of interest, larvæ and pupæ only being sought.

The Anopheles' larva is distinguished by the flat or parallel position normally assumed at the surface of the water, since during a great part of its existence it feeds at the surface and descends only when disturbed, but quickly rises to the top. The full-grown larva frequently descends and feeds below the surface.

Anatomically, the head of the larva is narrower than the thorax. A stump-like respiratory siphon projects from the eighth abdominal segment. The larva of other species of mosquitoes has a longer respiratory siphon, so that when it rises to the surface of the water to breathe, the body is submerged and usually appears to be hanging with the head downward. (See figs. 4, 5, and 6.)

The larvæ may be found variously colored white, yellow, brown, green, or black, the color sometimes depending upon the character of their food. The size varies with the age, the smallest being about one-sixteenth inch in length, while those reaching the stage before pupating (eight to nine days) are about one-fourth inch in length.

The Anopheles pupæ are comma-shaped in appearance. They are not so easily distinguished from other mosquito pupæ, the only reliable differences being in the arrangement of some of the minute hairs. Ordinarily in the surveys where Anopheles and the common Culex have been found, the Anopheles pupæ could be distinguished by the two breathing tubes located on either side of the back of the thorax, which appear funnel-shaped, with the broad end toward the surface, while those of the common Culex appear slender and narrow. (See fig. 7.)

The Anopheles (punctipennis, crucians, and quadrimaculatus) mosquito commonly met with is distinguished:

(1) By its position when resting on a surface. It holds its entire body in one straight line, forming an angle, or standing nearly vertical to the surface on which it stands.

(2) By its palpi, which are as long, or nearly as long, as the proboscis in both sexes. In the male the palpi are club-shaped apically, which distinguishes it from other male mosquitoes.

(3) By having dark spots on the wings. (There are other species of mosquitoes, as *Theobaldia incidens*, *Lutzia bigoti*, *Bancrofti* sp., etc., with spotted wings, but none was found in any surveys made.)

The male is recognized by the antennæ, which are densely covered with long hairs; in the female the hairs of the antennæ are short and fewer. Of course it is known that only the female bites, and is therefore the transmitter of the malarial parasites. (See figs. 8–15.)

For all practical purposes a knowledge of the general characteristic appearances of the larvæ and adult Anopheles is all that is necessary.

It has been found that even school children readily learn to distinguish Anopheles mosquitoes by these characteristics. The sanitary inspector on whom the local health officer may depend for discovering the breeding places of Anopheles soon becomes quite proficient.

Equipment.

The equipment necessary for making surveys consists of the following:

One small canvas bag.

One white enameled dipper, with hollow handle.

One stick for making extension to handle of dipper, and for beating a way through brush, briers, or tall grass.

One small glass dropper, with the tip cut off, so that the opening is about one-eighth inch in diameter. This to be used in securing larvæ and pupæ from the dipper.

Six small vials.

Six small pill boxes.

One note book and pencil.

One entomological bottle, 4 inches long, 1 inch in diameter (a large test tube will serve equally well), with cork. The bottom of the bottle is filled for a depth of 1 inch, with cotton and rubber bands saturated with chloroform. This is covered with a circular disk of cardboard or thin layer of cork. This is to serve as a catching bottle for collecting adult mosquitoes.

Method of Procedure.

For this survey one must be prepared to walk, as in no other way can the work be accomplished.

An ordinary *dipper* is used in making the examination of any collection of water. It is useless to dip in an open stream of running water. The dipper should be used for skimming the surface of water close to banks, shallow pools, or any collections of water, particularly where leaves, stumps, grass, trees, or any other vegetation are present. Another method may be employed by lowering the dipper into the water and allowing the water to rush over the edge. In any case, when a dipper of water is obtained it is examined for the presence of larvæ and pupæ.

If it is desired to preserve Anopheles larvæ and pupæ, these may be easily removed from the dipper with the dropper and placed in collecting bottles or other containers at hand. The convenience of using a dropper is that only the Anopheles larvæ and pupæ desired may be secured, thus eliminating all other species that may be in the dipper of water.

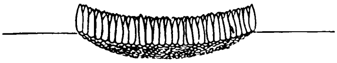

Fig. 1.—A raft of Culex ova. (After Deaderick.)

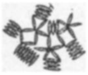

Fig. 2. — Patterns assumed by Anopheles ova. (After Deaderick.)

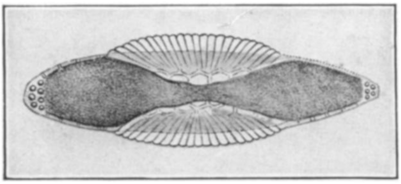

Fig. 3.—Egg, Anopheles maculipennis (quadrimaculatus). (After Ludlow.)

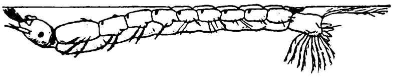

Fig. 4.—Larva of Anopheles mosquito. (Castellani and Chalmers. Modified after Howard.)

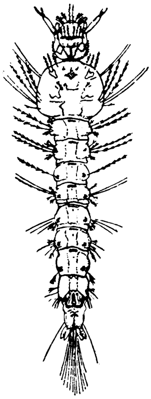

Fig. 5.—Larva of Anopheles maculipennis (quadrimaculatus). (Castellani and Chalmers, after Nuttall and Shipley.)

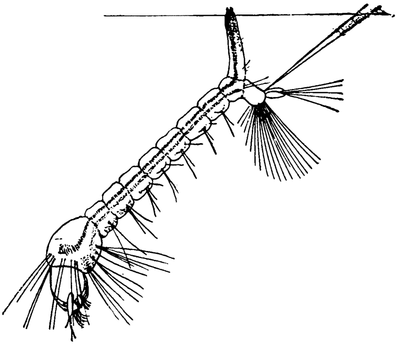

Fig. 6.—Larva of a Culex mosquito. (After Howard.)

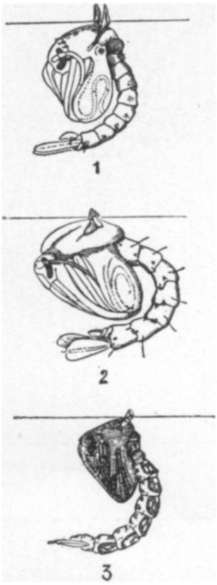

Fig. 7.—Pupae; 1 Culex; 2 Anopheles; 3 Aëdes calopus. (After Howard.)

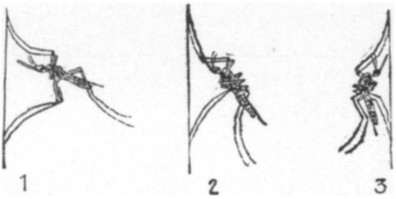

Fig. 8.—Resting posture of mosquitoes; 1 and 2 Anopheles; 3 Culex pipiens. (After Sambon.)

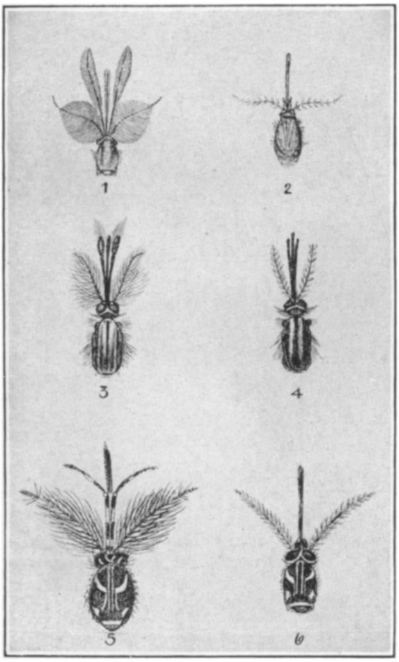

Fig. 9.—Heads of mosquitoes; 1 and 2 male and female Culex pungens; 3 and 4 male and female Anopheles; 5 and 6 male and female Aëdes calopus. (After Stitt.)

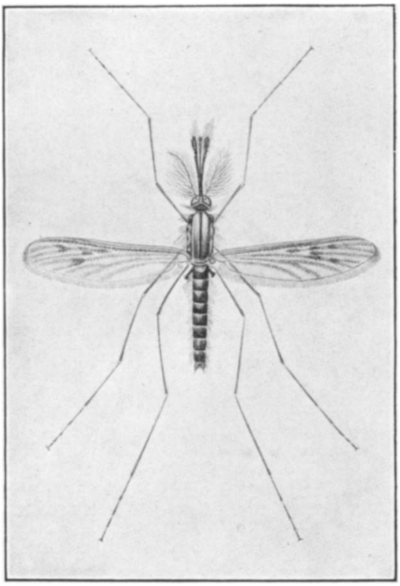

Fig. 10.—Anopheles maculipennis (quadrimaculatus), male. (After Castellani and Chalmers.)

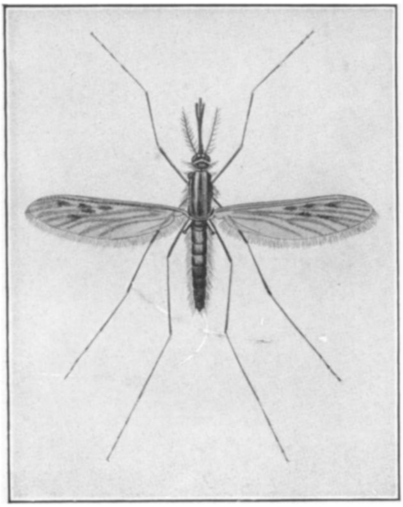

Fig. 11.—Anopheles maculipennis (quadrimaculatus), female. (Castellani and Chalmers, after Austen.)

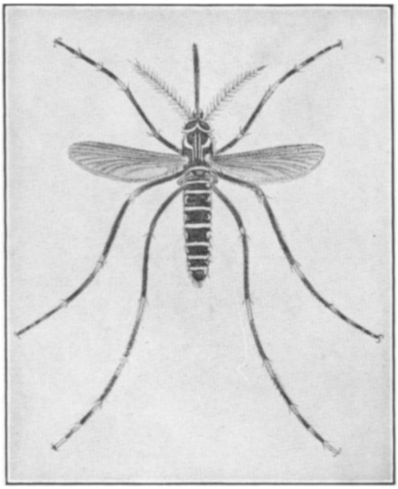

Fig. 12.—Aëdes calopus, female.

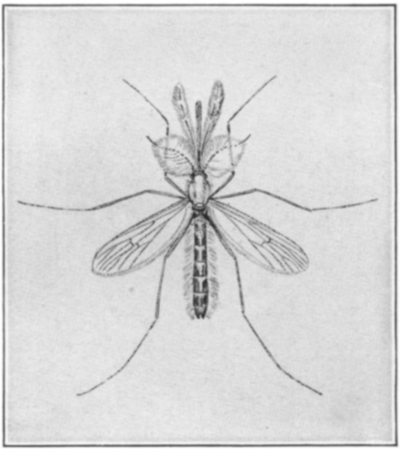

Fig. 14.—Culex pungens, male. (After Howard.)

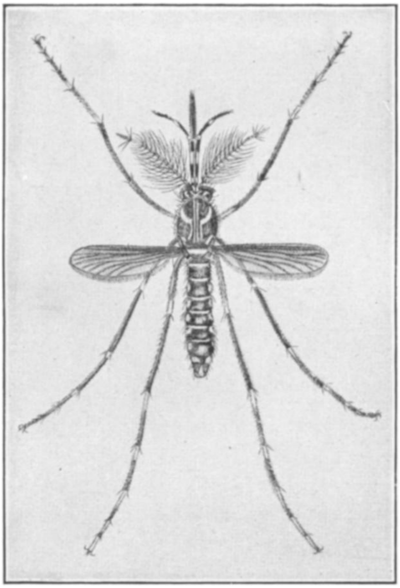

Fig. 13.—Aëdes calopus, male.

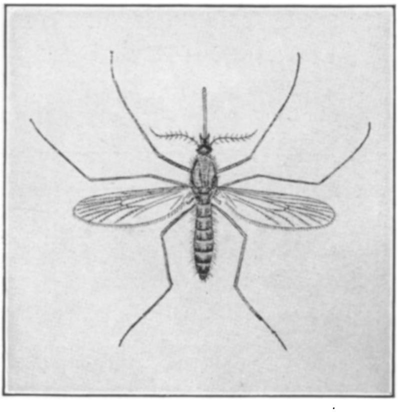

Fig. 15.—Culex pungens, female. (After Howard.)

A small label, giving the place and date of collection, may be stuck in with the cork.

It is important to note the number of larvæ and their apparent ages as shown by their comparative sizes.

Other conditions should be noted, such as the character of water, whether clean or muddy, stagnant, or flowing, the presence of vegetation, algæ, fish, water insects, and the like.

The presence of mosquito larvæ in any place examined is not always positive evidence that Anopheles are propagating there. For example, if all the larvæ found are small or not beyond the half-grown size, this may be due to adverse conditions, such as the presence of fish which eat the larvæ before they reach adult size; again, if only large or nearly grown larvæ are found, these may have been carried to the place by heavy rains. In a typical breeding place, larvæ in various sizes and stages will be found.

At times one will find what appears to be a most favorable place for the breeding of Anopheles and yet no Anopheles mosquito larvæ are to be found. A careful examination may explain this, e. g., presence of top-minnows, predaceous water insects such as "water boatmen," water shield, or other plants forming a dense mat over the water surface. The absence of larvæ may also be due to some chemical influence from waste discharges, such as dye water, coal-gas water, engine oils, etc., as may be evidenced by the presence of dead algæ or dead grass.

Breeding Places.

A general division of breeding places found may be given under two heads, viz:

(1) Natural or breeding places of selection or choice.

(2) Artificial or breeding places of necessity, opportunity, or accident.

The natural or breeding places of choice are: Brooks, creeks, branches, edges of ponds, ditches, street gutters, swamps, low wet areas, hog wallows, water-filled hoof prints of cattle, road pools in roads little traveled, pools among rocks, rice fields, etc.

It has been found that water from ice plants, exhaust water from steam plants, leaks from water pipes, mains, fire plugs, pumps, hydrants, overflows from troughs and tanks, in fact, any puddles of clear, fresh water collecting in places where grass, algæ (frog grass), or any other form of vegetation is present, form conditions which may be classed as natural breeding places, and are frequently prolific sources of Anopheles mosquitoes in many communities.

The artificial breeding places are barrels, hollow stumps of trees, boats, fountains, and the like. Wherever artificial containers have been found to contain Anopheles larvæ, natural breeding places were usually not very distant, or Anopheles mosquitoes were quite numerous, so that the selection of such places by them for laying their eggs seems to be one of convenience.

Artificial water containers may, therefore, at times and under certain conditions, become factors in breeding Anopheles mosquitoes, and will have to be dealt with when undertaking antimosquito measures.

Special Conditions.

A description of some of the special conditions encountered may be of interest.

Anopheles larvæ are rarely found in water containing sewage, or waste water containing soap, grease, or oil, but Culex mosquito larvæ are commonly encountered in such contaminated waters.

At Wynne, Ark., there is a ditch beginning at the railroad station, receiving the effluent water from a septic tank, and, a short distance from this, water containing lime from the railroad water-purification tanks.

The ditch takes a tortuous route for the first quarter of a mile of its course. In this the water was foul and Culex mosquitoes were breeding in enormous numbers; but in the remaining portion as it continued along a street gutter for a distance of a mile, the water, as a result of sedimentation and other influences, became clear and apparently clean. Here Anopheles found a most favorable breeding ground.

At Osceola, Ark., the overflow water from the septic tank of a hotel, discharging into a ditch, contained countless numbers of Culex larvæ and pupæ in the first 300 feet of its course, but Anopheles larvæ in the remaining portion of the ditch where the water was clean and clear.

At Forest City, Ark., a ditch receiving water from an ice plant and bottling works contained Anopheles and Culex larvæ, while at the place of juncture with the water from a discharging sewer no more Anopheles larvæ could be found, but Culex prevailed.

At Columbus, Ga., Senior Surg. H. R. Carter found a ravine running about 3 to 3½ miles to the river. In the upper end a sewer emptied into it. On the side of this ravine there were a number of small springs 50 to 200 feet distant, nine of them within a mile and a quarter. Along the course of the stream there were many marshy places containing clear and apparently clean water from these springs. Anopheles mosquitoes in great numbers were breeding up to the points where these emptied into the foul stream of the ravine. No Anopheles were to be found in the polluted stream until about a mile and a quarter below its origin, where the water became clear and odorless and flowed broadly over a grass-covered area for a quarter of a mile, at which point sewage water again entered and Anopheles again disappeared. Culex larvæ in enormous numbers were found in the foul waters.

At Greenville, Miss., a ditch received waste water from a municipal pumping plant. Beginning at the source for a distance of about 300 feet no mosquito life was found, due to the engine oil which covered the water; then for a distance of about two squares—600 feet—only Culex larvæ and pupæ were found, increasing proportionately farther on where the ditch was deep and covered with vegetation.

In only one place, at Wakefield, Va., were Anopheles larvæ found in a pool of water which was soapy in character. It is possible that the water contamination occurred after the larvæ had developed.

Collections of water formed from steam exhausts and waste water have been mentioned as frequently contributing to the formation of breeding places for Anopheles mosquitoes. The examination of industrial and manufacturing plants, such as ice plants, mills, artesian wells, electric light and power plants, lumber mills, bottling works, and many manufacturing concerns, in places where malaria prevails, is therefore important. In every State where surveys were made, as in Arkansas, Mississippi, South Carolina, North Carolina, and Virginia, some industrial plants were responsible for the formation, in close proximity to habitations, of breeding places of Anopheles mosquitoes.

Many artificial water containers were examined and Anopheles found in some of them. Some of these observations are interesting.

At Lake Village, Ark. (1913), two barrels containing rain water, were found standing beside a house. In one of them Culex and Aëdes (Stegomyia) were breeding and in the other Anopheles, Culex, and Aëdes. The only difference noted in the conditions of the two barrels was that the barrel having Anopheles larvæ contained dead leaves from trees.

On a trestle at Helena, Ark., there were three open barrels containing water with no vegetation, partly covered with cinders and coal dust. Anopheles larvæ were found in them.

Barrels containing water for fire protection purposes, as in lumber yards, were frequently found breeding Culex and Aëdes (Stegomyia) mosquitoes, but occasionally Anopheles were also found.

At Osceola, Ark., a garden fountain, containing large water lilies, was found to be swarming with Anopheles larvæ and pupæ. It was learned that a catfish, when small, was placed in this fountain and as it grew to full size had disposed of a number of small gold fish. Gold fish ordinarily feed on larvæ.

No bottles or tin cans were found (in 1914) which contained Anopheles larvæ. In 1911 at Mobile, Ala., one tin can and one bottle having grass in them were found to contain Anopheles larvæ.

Other interesting artificial breeding places of Anopheles were found, such as a boat used as a watering trough, at Scott, Miss.; a partly submerged boat in the river at Emporia, Va.; the hollow stump of a burned oak tree at Scott, Miss.; an iron pot located 20 feet from a stream, standing beside other artificial water containers, tubs, and barrels, at Helena, Ark.

Prevailing Species of Mosquitoes.

It is important that the prevailing species of Anopheles mosquitoes be known. This may be ascertained in two ways:

(1) By collecting the adult mosquitoes.

(2) By breeding out larvæ and pupæ.

In breeding out larvæ the adult or nearly full grown larvæ should be selected. Pupæ will hatch out in one or two days.

For making a collection of mosquitoes a collecting bottle, such as an entomological bottle, or a large-mouth test tube, previously described under equipment, is very convenient.

When chloroform is not available the bottle or test tube may be charged by blowing the smoke from a cigar, pipe, or cigarette into it. The smoke leaves a minute deposit of nicotine, which, while a little slower in its action in killing mosquitoes collected, will serve for a day or two.

Searching for the Adult Anopheles Mosquitoes.

The places where Anopheles are likely to hide during the daytime are sought. The search often requires considerable time and patience, as this mosquito is very shy and dislikes daylight.

The places where adult Anopheles were collected were garrets, bedrooms, on walls, under mosquito bars, behind pictures, on clothing, behind doors and furniture, in barns, open fireplaces, privies, chicken coops, wood and coal sheds, stables, garages, under porches and buildings, in wind-protected corners of porches, in empty barrels, trash heaps, wagons, carriages, automobiles, on trunks of trees, in the hollows of trees and tree stumps, in caves, eroded overhanging banks of streams, and on spider webs.

Since Anopheles are frequently, almost invariably, to be found in the outhouse privies, often engorged with blood, the question may arise if these places may not be a source of malarial infection. The sanitary privy should therefore be made mosquito proof as well as fly proof.

Anopheles mosquitoes are undoubtedly carried great distances, as they are not infrequently found in carriages, on tops of wagons and automobiles, and in freight cars.

Spider webs and cobwebs are favorite resting places for Anopheles mosquitoes, from which they will fly upon the slightest disturbance.

The Anopheles appear hanging from a web, and as many as 60 have been counted on a single web.

Records and Other Data.

A record of all mosquito-breeding places found should be kept. All mosquito-producing areas, such as pools, ditches, streams, etc., should be marked on a map (to be known as a mosquito-campaign map) and each place given a number.

The date when each place is treated or inspected should also be recorded, so that every step may be followed, particularly in checking up the work done by inspectors or others.

The record of the different measures taken and the cost of each, will also be valuable for making comparative studies of results of antimosquito work.

Antimosquito Measures.

The measures generally employed are, briefly, the following:

(a) Regrading and training of streams, creeks, or similar natural watercourses so as to favor a free current.

The shallow grass-grown margin of streams, ponds, or any bodies of water must be cleared and the banks made with a clean-cut edge in order that any top-feeding minnows present may have a clear field for their activity and that this field may be extended.

(*b*) *Drainage* is employed for the removal of standing water, or to produce a movement of water unfavorable to mosquito breeding. Ditches should be as few and as short as possible, and so constructed that any water present will be confined to a narrow channel. Open ditches must be kept free of grass, débris, or any other obstructions. They may be made permanent and easy of maintenance by lining with cement, stone, or wood.

Ditches, as ordinarily used, should have a bottom not over 8 inches wide and the sides sloping.

Subsoil tile drains, while more costly, are most effective.

(*c*) *Filling* in of low places that are too low to drain, or which can not be drained economically. For this purpose any available porous material, such as ashes, sawdust, or shavings, may be employed. When using sawdust or shavings, such material should extend 6 inches or more above high water which follows a heavy rain.

(*d*) *Oiling and larvacides.*—Oil may be applied by the use of:

(1) Garden watering pot.

(2) Knapsack sprayer.

(3) Drip can for intermittent or continuous oiling regulated to deliver 18–20 drops of oil per minute. The bottom of the drip can should be about 4 feet above the level of the water surface.

(4) Oil-saturated bundle of cotton waste. This is to be anchored into place and will serve for about one week.

Larvacides are used as a substitute for oil. The most effective larvacide in use in Panama is a phenol preparation. At some places, industrial waste discharges, such as dye water, coal-gas water, and bleaching water, may be economically diverted into a stream or small watercourse and serve every purpose of a larvacide.

(*e*) *Natural enemies:* Stocking with top-feeding minnows is a measure applicable in certain ditches, ponds, pools, swamps, streams, and many other bodies of water.

General Remarks.

Constant supervision and inspection must be made of all antimosquito work undertaken, and new breeding places must be sought.

The season of mosquito prevalence begins in the Southern States in March and April and continues until November. Antimosquito measures should be planned and begun in the winter months, but much depends upon the character and extent of the work to be done and the climatic conditions. The work should begin from the center of a town and extend, for practical purposes, a quarter of a mile or more beyond the corporate limits or bordering residences to be benefited.

No antimalarial campaign, however, will or can be successfully and economically conducted without making surveys on the lines given.

It has been the experience of all the officers engaged in this work that antimalarial work is more readily undertaken by communities in which the majority of the population is engaged in a few industries, or a single large industry, than in the ordinary commercial towns having divided interests.

There are several reasons for this, namely:

(1) Managers of large industries are accustomed to finance undertakings from which no immediate returns may be obtained.

(2) Housing, sanitation, and the medical service for all residents are provided, and are therefore under their direct control.

(3) Steady, sufficient, and efficient labor is assured when living conditions are made attractive and healthful.

In the ordinary commercial towns, public interest must be aroused in order to create a demand for health work. An educational campaign must be conducted, and the people taught that the disease is preventable. It is an asset to any town to advertise its freedom from malaria, particularly in the Southern States.